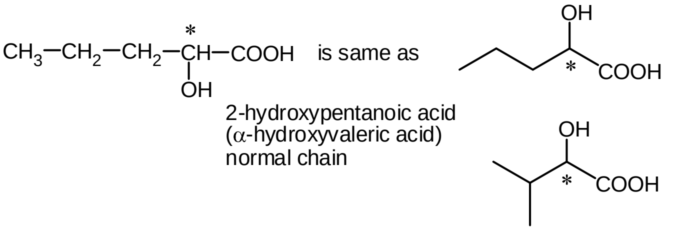
№ 31. HYDROXYCARBOXYLIC ACIDS. CHARACTERISTICS, CLASSIFICATION, ISOMERISM, PROPERTIES. REPRESENTATIVES

I. Characteristics and classification. Some biochemically important low molecular weigh compounds belong to the class of hydroxycarboxylic acids (mono- or poly-). A hydroxy acid is a carboxylic acid substituted with one or more hydroxyl groups. Depending on the number of carboxylic and hydroxyl groups there are:

Monohydroxymonocarboxylic acids		**Polyhydroxypolycarboxylic acids**
HO—R—COOH	aliphatic hydroxy acids	$(HO)_n$—R—$(COOH)_m$
HO—Ar—COOH	aromatic hydroxy acids	$(HO)_n$—Ar—$(COOH)_m$

II. Isomerism. **1. Chain isomerism**. The main chain of the carboxylic acid may be straight (normal) or branched. When the branching carbon is at the end of the chain, the carboxylic acid is often named isocarboxylic acid. When writing the chains, zig-zag lines are used, where each "bending" point represents a -CH_2-group (methylene group). Multiple bonds, branching or heteroatoms in the chain cannot be omitted from such condensed formulas.

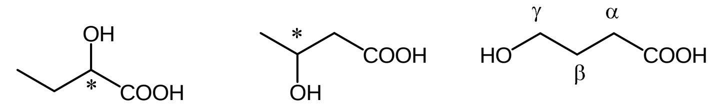


$CH_3-CH_2-CH_2-\overset{*}{C}H(OH)-COOH$

2-hydroxypentanoic acid (α-hydroxyvaleric acid) normal chain

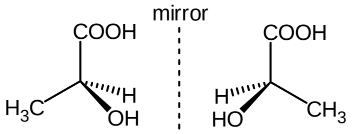
2-hydroxy-3-methylbutanoic acid (α-hydroxyisovaleric acid) branched chain

2. Positional isomerism. Hydroxycarboxylic acids that have the same carbon skeleton but differ in the position occupied by the hydroxyl group are called positional isomers. When Greek letters are used, instead of numbers in the nomenclature names, to show the location of -OH group, the first carbon atom of the carboxyl group is not counted.



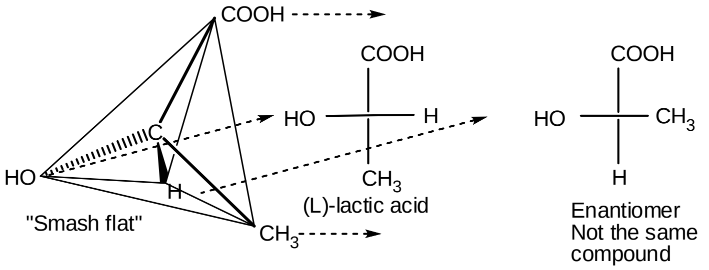
α-hydroxybutyric acid β-hydroxybutyric acid γ-hydroxybutyric acid

3. Optical isomerism. All α-hydroxybutyric (2-hydroxybutanoic), β-hydroxybutyric (3-hydroxybutanoic), α-hydroxyvaleric (2-hydroxypentanoic), α-hydroxyisovaleric (2-hydroxy-3-methylbutanoic) acids contain one carbon atom that is substituted with four different groups. This carbon is designated in the formulas by an asterisk *. Such carbon atom is called chiral. Molecules containing chiral atoms (they may not be only carbons) are called chiral molecules. The definition of "chiral" and the property "chirality" was given by Lord Kelvin in 1904 "*I call any geometrical figure, or group of points, chiral, and say it has chirality, if its image in a plane mirror, ideally realized, cannot be brought to coincide with itself.*" Presently, this statement is universally accepted as *the* definition of chirality (Greek word for hand, χειρ (cheir)). Other statements simply transpose it in other contexts, for instance, in chemistry when a molecule cannot coincide with its mirror image is called chiral molecule. Human hands are chiral objects. Stereoisomers that are related as an object and its nonsuperimposable mirror image are classified as **enantiomers**. Enantiomers behave identically in common chemical reactions but differ when the reaction is conducted in chiral environment (e.g. with the help of an enzyme, which is chiral molecule) and they interact differently with polarized light. This physical property is known as optical activity. Therefore enantiomers are called also optically active isomers. When the substances are not chiral, they are always optically inactive. A chiral substance is optically active if the amounts of its enantiomers present are not equal. Lactic acid (2-hydroxypropanoic acid) is one of the simplest examples where to show how to draw enantiomers on a flat page of paper, that is, how to express the different positions of groups in space as shown below:



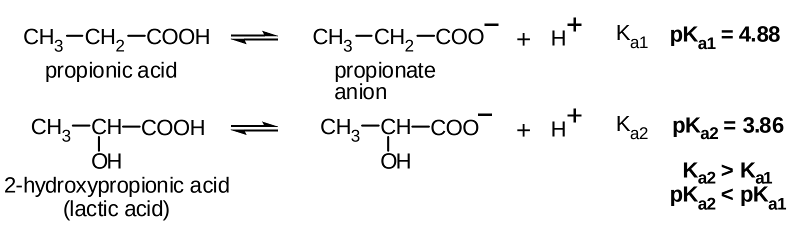
enantiomers of lactic acid

For this purpose **Fischer projections** are used. Fischer projections are always generated the same way: the molecule is oriented so that the vertical bonds at the chiral carbon are directed away from you and the horizontal bonds point toward you. Since these projections are related to glyceraldehyde and are



widely used for amino acids and carbohydrates, the most oxidized carbon is placed on the top of the projection. A projection of the bonds onto the page is a cross. The chiral carbon atom lies at the center of the cross but is not explicitly shown. Groups in Fischer projection cannot switch places because the result is enantiomer, and projection formulas cannot be rotated 90°, 270°, etc. but can be rotated at 180°.

III. Properties. **Acidity of hydroxycarboxylic acids**. Carboxylic acids are proton donors when dissolved in water. The strength of a carboxylic acid depends on the extent of its ionization: *the more ionized it is, the stronger it is*. They are typically weak acids, meaning that in aqueous solutions they are only partially dissociated into H^+ (hydroxonium cations) and $RCOO^-$ (carboxylate anions). For example, at room temperature, only 2 out of 10 000 propanoic acid molecules are dissociated in water. The highly polar O-H bond causes the hydrogen atom to be labile, thus it dissociates easily to give the H^+ ion. The carboxylate anion is relatively more stable than the acid because the negative charge is dissipated (delocalized) on both oxygen atoms. Additional electron-withdrawing atoms or groups, such as hydroxyl or halogens (the R-group) increase the acidity through inductive effects (experimentally observable effect of the transmission of charge through a chain of atoms by electrostatic induction). For example, trichloroacetic acid (three electron-withdrawing chlorine atons) is a stronger acid than lactic acid (one -OH group), which in turn is stronger than propanoic acid (no electronegative constituent). The higher acidity of lactic acid in comparison with propionic acid signifies that the -OH group acts as an electron-attracting group (it has negative inductive effect, –I). The inductive effect diminishes quickly with distance. As the induced by the substituent change in polarity is less than the original polarity, the inductive effect rapidly dies out, and is significant only over a short distance. As a rule, hydroxycarboxylic acids are stronger electrolytes than carboxylic acids with the same number of carbon atoms in their chains. This is expressed by lower pK_a values of the hydroxy acids.



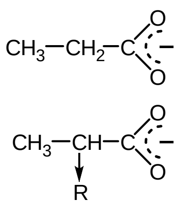
$$CH_3-CH_2-COOH \rightleftharpoons CH_3-CH_2-COO^- + H^+ \quad K_{a1} \quad pK_{a1} = 4.88$$

propionic acid; propionate anion

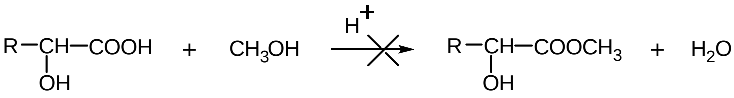
$$CH_3-\underset{OH}{CH}-COOH \rightleftharpoons CH_3-\underset{OH}{CH}-COO^- + H^+ \quad K_{a2} \quad pK_{a2} = 3.86$$

2-hydroxypropionic acid (lactic acid)

$$K_{a2} > K_{a1}$$
$$pK_{a2} < pK_{a1}$$

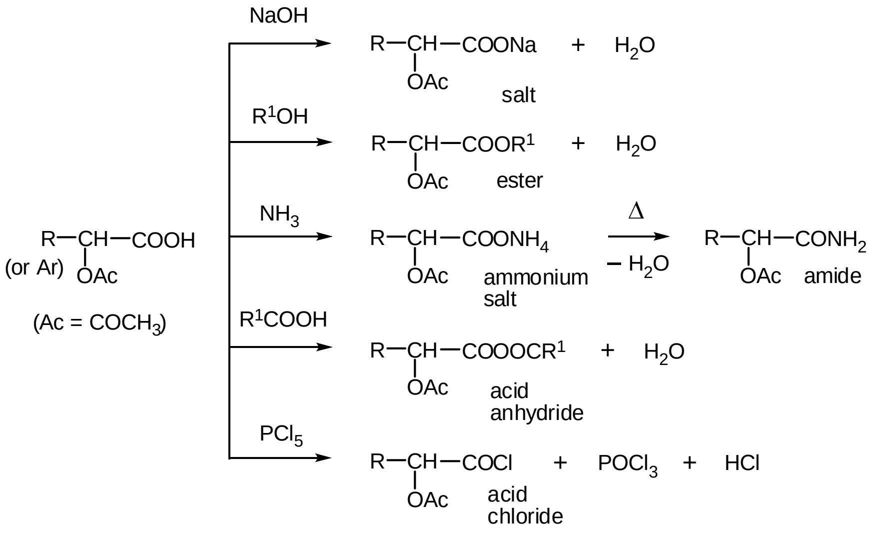


Chemical properties. Bifunctional derivatives can in principle react in chemical reactions with both functional groups. This makes some simple reactions to proceed in several ways, e.g. direct esterification with methanol of an α-hydroxy acid, because its -OH group will react the same way as methanol. The strategy that is routinely followed is to *protect* one functional group during the reactions with which it is incompatible and then to *remove* the protecting group. A **protecting group** or protective group is introduced into a molecule by chemical modification of a functional group in order to obtain chemoselectivity in a subsequent chemical reaction. It plays an important role in multistep organic synthesis of delicate compounds. Typical protecting group for an alcohol is the acetyl group (removable by acid or base catalyzed hydrolysis) and for a carboxylic acid is the ester group (methyl ester – removable by hydrolysis, benzyl ester – by hydrogenolysis or *tert*-butyl ester – by acid).



$$R-\underset{OH}{CH}-COOH + CH_3OH \xrightarrow[\times]{H^+} R-\underset{OH}{CH}-COOCH_3 + H_2O$$

The reactivity of the hydroxyl group in hydroxy acids is suppressed by converting it into ester, if only modification of the carboxyl group is sought. Such examples of chemical transformations of an α-hydroxy acid whose -OH group is protected with acetyl group are:



$$\underset{OAc}{R-CH}-COOH \ \text{(or Ar)}, \quad (Ac = COCH_3)$$

$$\xrightarrow{NaOH} R-\underset{OAc}{CH}-COONa + H_2O \quad \text{salt}$$

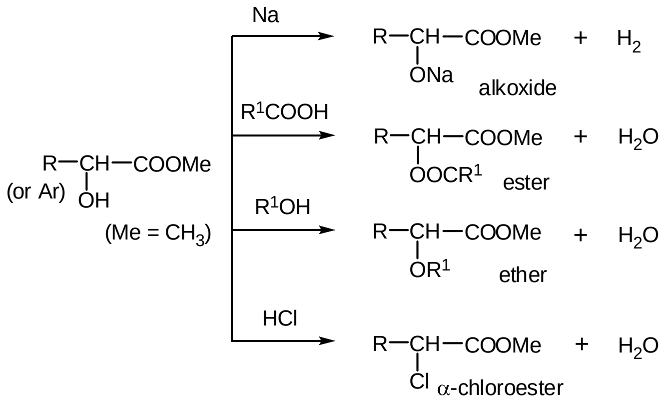
$$\xrightarrow{R^1OH} R-\underset{OAc}{CH}-COOR^1 + H_2O \quad \text{ester}$$

$$\xrightarrow{NH_3} R-\underset{OAc}{CH}-COONH_4 \ \text{ammonium salt} \xrightarrow[-H_2O]{\Delta} R-\underset{OAc}{CH}-CONH_2 \ \text{amide}$$

$$\xrightarrow{R^1COOH} R-\underset{OAc}{CH}-COOOCR^1 + H_2O \quad \text{acid anhydride}$$

$$\xrightarrow{PCl_5} R-\underset{OAc}{CH}-COCl + POCl_3 + HCl \quad \text{acid chloride}$$

When the carboxyl group is protected as methyl ester (Me), the following reactions are possible:



$$\underset{OH}{R-CH}-COOMe \ \text{(or Ar)}, \quad (Me = CH_3)$$

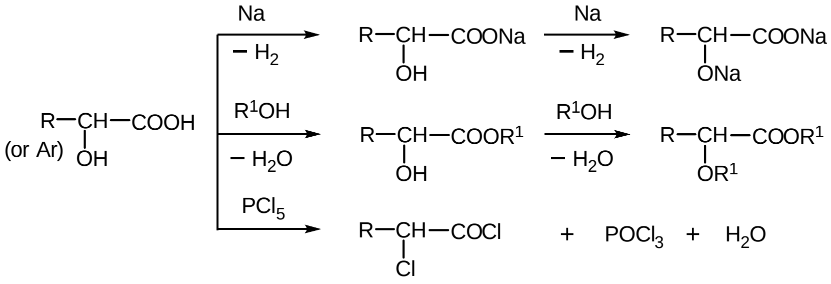
$$\xrightarrow{Na} R-\underset{ONa}{CH}-COOMe + H_2 \quad \text{alkoxide}$$

$$\xrightarrow{R^1COOH} R-\underset{OOCR^1}{CH}-COOMe + H_2O \quad \text{ester}$$

$$\xrightarrow{R^1OH} R-\underset{OR^1}{CH}-COOMe + H_2O \quad \text{ether}$$

$$\xrightarrow{HCl} R-\underset{Cl}{CH}-COOMe + H_2O \quad \alpha\text{-chloroester}$$

If both functional groups are free (unprotected) then certain reactions can proceed step-wise.



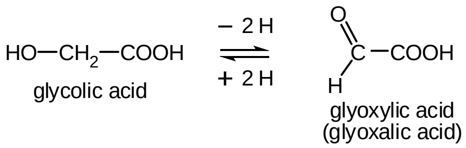
$$\underset{OH}{R-CH}-COOH \ \text{(or Ar)}$$

$$\xrightarrow[-H_2]{Na} R-\underset{OH}{CH}-COONa \xrightarrow[-H_2]{Na} R-\underset{ONa}{CH}-COONa$$

$$\xrightarrow[-H_2O]{R^1OH} R-\underset{OH}{CH}-COOR^1 \xrightarrow[-H_2O]{R^1OH} R-\underset{OR^1}{CH}-COOR^1$$

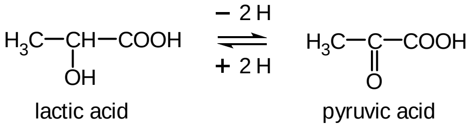
$$\xrightarrow{PCl_5} R-\underset{Cl}{CH}-COCl + POCl_3 + H_2O$$

Oxidation reactions Hydroxycarboxylic acids are oxidized more easily than the unsubstituted aliphatic counterparts which are more stable towards oxidizing agents (with exception of formic acid). The reason for this behavior is the presence of hydroxyl group. Depending on the position of this group and the type of carbon connected to it (primary, secondary, tertiary), the resulting from oxidation products are formyl (aldehyde) acids or keto acids.



$$\underset{\text{glycolic acid}}{HO-CH_2-COOH} \underset{+2\,H}{\overset{-2\,H}{\rightleftharpoons}} \underset{\text{glyoxylic acid (glyoxalic acid)}}{H-\overset{O}{\overset{\|}{C}}-COOH}$$

Glycolic acid (or hydroxyacetic acid) is the smallest α-hydroxy acid. This colorless, odorless, and hygroscopic crystalline solid is highly soluble in water. It is used in various skin-care products because it can penetrate skin easily and improves appearance by chemical peel process. Glycolic acid can be obtained by S_N2 substitution of chlorine in chloroacetic acid. $Cl-CH_2-COOH + NaOH \longrightarrow HO-CH_2-COOH + NaCl$



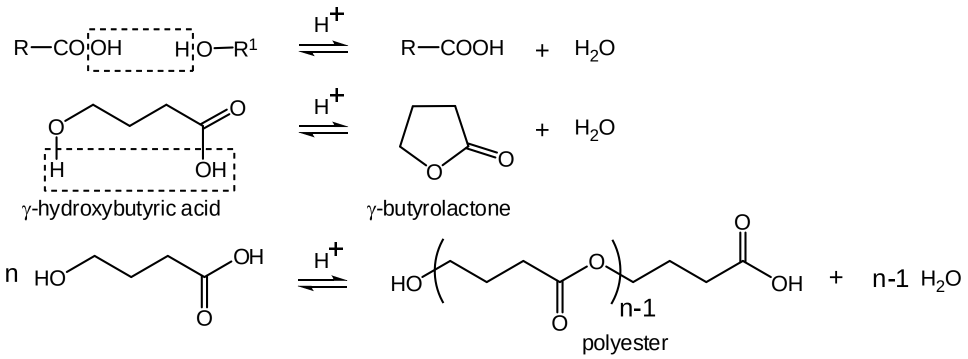
$$\underset{\text{lactic acid}}{H_3C-\underset{OH}{CH}-COOH} \underset{+2\,H}{\overset{-2\,H}{\rightleftharpoons}} \underset{\text{pyruvic acid}}{H_3C-\underset{O}{\overset{\|}{C}}-COOH}$$

lactate-pyruvate redox pair

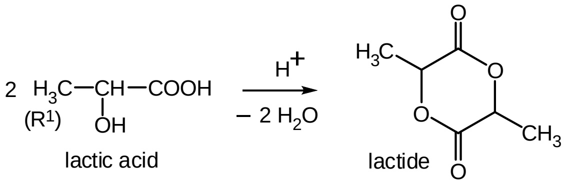
Lactic acid is oxidized to pyruvic acid. The biochemical process involves removal of two hydrogen atoms from lactic acid, and is catalyzed by the enzyme lactate dehydrogenase.

Oxidation describes the *loss* of electrons (loss of hydrogen or *gain* of oxygen). In biological systems is common to think of simultaneous loss of 2 electrons and 2 protons = 2 hydrogen atoms as an oxidation process. At physiological pH acids exist as anions, therefore it is common in biochemistry to use the corresponding names of their salts, e.g. lactate-pyruvate redox process, catalyzed by lactate dehydrogenase.

Esterification reactions - intermolecular and intramolecular.

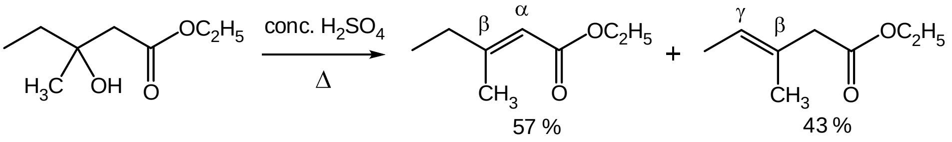


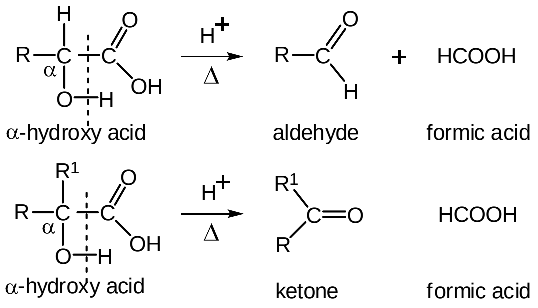
Carboxylic acids react with alcohols under acid catalysis to yield esters. Such reaction is intermolecular, because it proceeds between two molecules. A hydroxy acid contains both functional groups that are involved in the reaction: -COOH and -OH. For this reason hydroxy acids can undergo intramolecular (within one molecule) esterification to yield cyclic esters. Such cyclic esters are named, according to the nomenclature, lactones. Lactonization, like normal esterification, is an equilibrium process. The most stable lactones have five- or six-membered rings, therefore there is a substantial amount of lactone present at equilibrium. γ-Lactones (five-membered ring) are so stable that, in the presence of dilute acids at room temperature, 4-hydroxy acids (R-CH(OH)-$(CH_2)_2$-COOH) immediately undergo spontaneous lactonization (cyclization). β-Lactones are less stable. Naturally, during the lactonization, a competing reaction is the intermolecular esterification which gives polymeric esters. The principle of high dilution is applied to maximize the lactone amount, in order to avoid intermolecular esterification giving polyester (industrially some polyesters are obtained by ring-opening polymerization of a lactone). Industrial polyesters have achieved very wide use in modern life. Fabrics woven from polyester thread or yarn are used extensively in apparel and home furnishings, from shirts and pants to jackets and hats, bed sheets, blankets and upholstered furniture. This development is mostly due to the fact that hydroxy acids can form also intermolecular esters with each other.



α-Hydroxy acids cannot form stable lactone ring (three-membered). Nevertheless, two molecules of them easily condense under acid catalysis. The initial dimeric intermediate (a monoester with open chain) is a form of 5-hydroxyacid, so lactonization can occur. The cyclic dimeric product, containing two ester moieties, is called lactide.

In strongly acidic medium hydroxy acids can loose molecule of water – reaction called dehydration. β-Hydroxy acids and their derivatives can dehydrate to two possible products: α,β- and β,γ-unsaturated products. Since the conjugation with an ester group is less effective than with an aldehyde group, a mixture of α,β- and β,γ-unsaturated products is obtained. On heating in acidic medium, α-hydroxy acids give carbonyl compounds, aldehydes or ketones, and formic acid.





Representatives I. Monohydroxy monocarboxylic acids

Carbonic acid (H_2CO_3, HO-COOH) is an inorganic compound formed in an equilibrium reaction of CO_2 dissolved in water. Only about 1% of the carbon dioxide dissolved in water exists as carbonic acid. Carbonic acid is weak diprotic acid which plays an important role in respiration and

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
$$H_2CO_3 + H_2O \rightleftharpoons HCO_3^- + H_3O^+ \qquad K_a = 4.3\text{x}10^{-7}$$
$$HCO_3^- + H_2O \rightleftharpoons CO_3^{2-} + H_3O^+ \qquad K_a = 5.6\text{x}10^{-11}$$

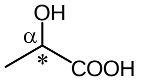
in acid-base balance of the blood. (The cited constant $K_a = 4.3\text{x}10^{-7}$ is for CO_2/HCO_3^-). Carbonic acid is an intermediate step in the transport of CO_2 out of the body via respiratory gas exchange. The hydration reaction of CO_2 is catalyzed by the enzyme **carbonic anhydrase** (in red blood cells) which both increases the reaction rate and dissociates a hydrogen ion (H^+), leaving bicarbonate (HCO_3^-) dissolved in the blood plasma. This catalyzed reaction is reversed in the lungs, where the enzyme converts the bicarbonate back into CO_2 and allows it to be expelled. Carbonic acid also plays a very important role as a buffer in mammalian blood. The equilibrium between carbon dioxide and carbonic acid is very important for controlling the acidity of body fluids.

$$CO_3^{2-} + 2\,H_3O^+ \rightleftharpoons H_2CO_3 + 2\,H_2O$$
$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

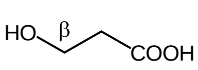
Strong acids react with carbonates to produce CO_2 from initially formed carbonic acid. Any acid that is stronger than carbonic acid reacts with carbonates to produce carbon dioxide gas. Insoluble carbonates such as calcium carbonate also react with strong acids.

$HO—CH_2—COOH$
glycolic acid

Glycolic acid is found in some sugar-crops such as cane-sugar juice, sugar beet, and sour green fruits.

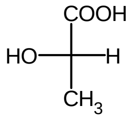


lactic acid

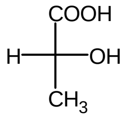


β-hydroxypropionic acid

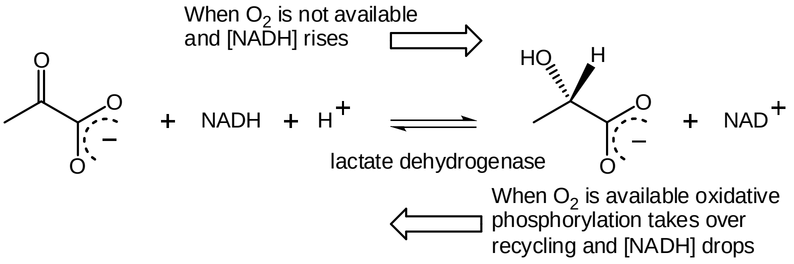
Hydroxypropanoic acids Two such acids are the positional isomers: α-hydroxypropionic and β-hydroxypropionic acids. The trivial and more often used name of α-hydroxypropionic acid is **lactic acid** (*acidum lacticum*). Lactic acid has two enantiomers designated as (L)- and (D)-. The L-(+)-lactic acid is the biochemically important enantiomer. In animals, L-lactate is constantly produced by reduction of pyruvate (final product of glycolysis of glucose) via the enzyme lactate dehydrogenase (LDH) in normal metabolism and exercise. When oxygen is absent or in short supply, e.g. during running or exercising, when the demand for energy is high, lactate is produced faster than the ability of the tissues to remove it and lactate concentration begins to rise. This is a beneficial process since the regeneration of NAD^+ from NADH (lactic acid fermentation) ensures that energy production is maintained and exercise can continue. Contrary to popular belief, this increased concentration of lactate does not directly cause acidosis, nor is it responsible for delayed onset muscle soreness. Low oxygen conditions occur in animal muscles when the rate of working exceeds the rate of oxygen supply, e.g. when sprinting.



L-(+)-lactic acid
(*S*)-

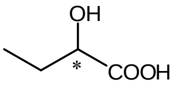


D-(-)-lactic acid
(*R*)-

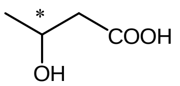


The D-(-)-lactic acid is formed by the action of certain bacteria on lactose (milk sugar) and it is responsible for the characteristic odor and the taste of sour milk. This acid is found in fermented dairy products (the result is curdling of casein). Consumption of products containing (D)-lactic acid should be avoided.

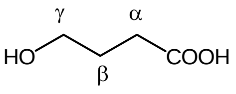
Three positional isomers are possible for **hydroxybutyric acids**. **α-Hydroxybutyrate** is produced in mammalian hepatic tissues that catabolize L-threonine or synthesize glutathione.



α-hydroxybutyric acid



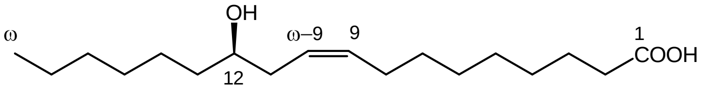
β-hydroxybutyric acid



γ-hydroxybutyric acid

β-Hydroxybutyrate is synthesized in the liver from acetyl-CoA and it is one of so called ketone bodies (along with acetoacetate and acetone), which levels are raised in ketosis. A blood test for β-hydroxybutyrate on diabetic patients may show the presence of ketones which may be associated with diabetic ketoacidosis. It can also be used for the synthesis of biodegradable plastics.

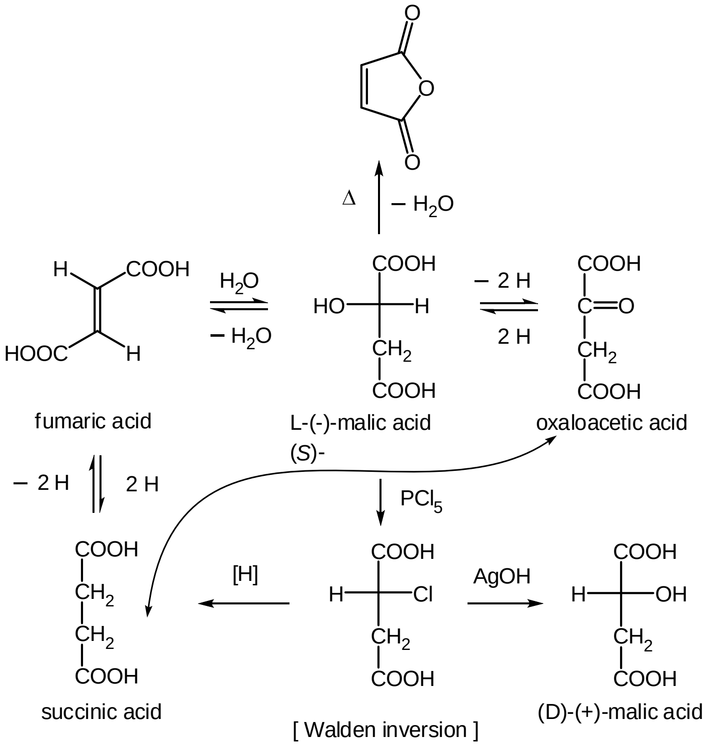
γ-Hydroxybutyrate is a naturally-occurring substance found in the central nervous system, wine, beef, small citrus fruits, and almost all animals in small amounts. The acid abbreviated GHB is categorized as an illegal drug in many countries. GHB is a CNS depressant used as an intoxicant, often in clubs, discotheques to enhance the experience on the dance floor. Some athletes and bodybuilders also use GHB to elevate human growth hormone *in vivo*.



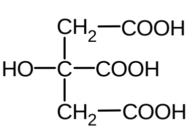
Ricinoleic acid ((R)-12-hydroxy-9-*cis*-octadecenoic acid) is an unsaturated omega-9 fatty acid that naturally occurs in plants and is the main ingredient of castor oil. Ricinoleic acid exerts remarkable analgesic and anti-inflammatory effects. The zinc salt is used in personal care products, such as deodorants. **ω-Hydroxy acids** – naturally-occurring are C16 (ω-OH palmitic) and C18 (ω-OH stearic) which are key monomers of cutin in plant cuticle (waxes).

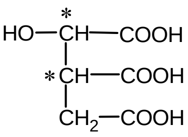
II. Polyhydroxy polycarboxylic acids. The combination of several hydroxyl and carboxyl groups is common in naturally occurring compounds. Many hydroxy acids are important in Nature and have trivial names: malic acid, citric acid, and tartaric acid. **Malic acid** (hydroxysuccinic acid, *acidum malicum*) is found in unripe fruits, fruit juices, sour or tart food. The L-(-)-enantiomer is the natural compound. Salts and esters are called malates. (They should not be confused with maleic (maleates) acid.) The interconversions succinate / fumarate, fumarate / malate, malate / oxaloacetate are biochemically relevant reactions. The synthetic relation of L-(-)-malic acid with D-(+)-malic acid includes inversion because of a back attack in S_N2 reaction of chloride on the chiral carbon carrying –OH.

Citric acid and malic acid are intermediates in the citric acid cycle (called also Krebs cycle).



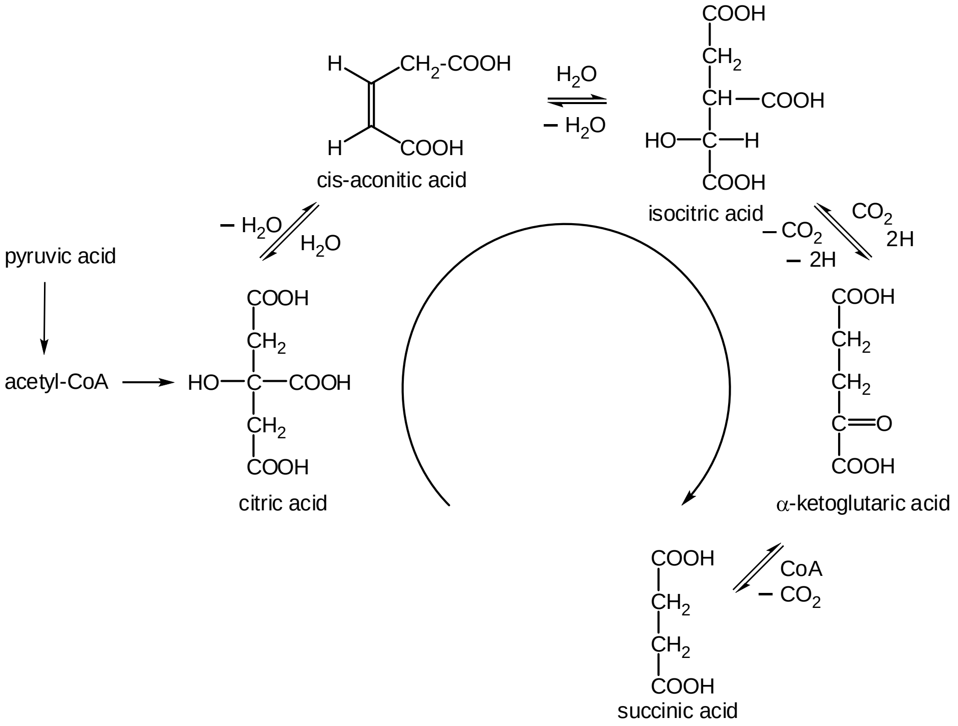
Citric acid (citrates) is hydroxy tricarboxylic acid found in citrus fruits. Lemons and limes have particularly high concentrations of the acid, as much as 8% of the dry weight. It is a natural preservative and is also used to add an acidic, or sour, taste to foods and soft drinks.


citric acid


isocitric acid

In **isocitric acid**, the alcohol group is switched from a tertiary carbon that cannot be oxidized, into secondary carbon that can be oxidized. Isocitric acid contains two chiral carbon atoms (*) whereas citric acid does not have any.

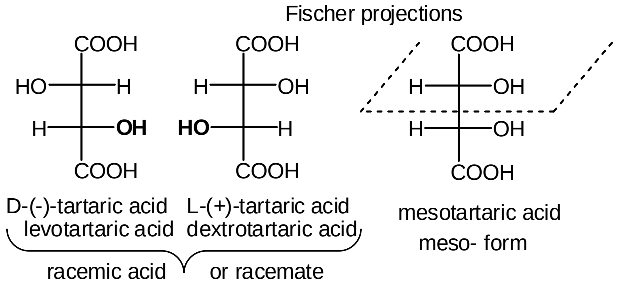
In biochemistry, citric acid is the beginning stage of mayor cyclic route of carbohydrate oxidation. Citric acid is the first of tricarboxylic acids, formed by condensation of pyruvate with oxaloacetate, therefore the cycle is known also as citrate cycle or simply, Krebs cycle (tricarboxylic acids' cycle).



Partial sequence of reactions in the cycle is: 1. the acetyl group of pyruvate, via acetyl-CoA, is added to oxaloacetate to give citrate; 2. citrate is dehydrated to *cis*-aconitate (this is dehydration of an alcohol); 3. water adds to the double bond to yield isocitrate; 4. the secondary now –OH group is oxidized (dehydrogenated) to oxalosuccinate (not shown on the left); 5. oxalosuccinate loses a carboxyl group (decarboxylates) to give α-ketoglutarate. α-Ketoglutarate undergoes a series of reactions that include coenzyme A and another decarboxylation to give succinate.

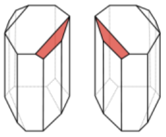
From chemical perspective the cellular respiration at molecular level involves several basic points:

- Two carbon atoms enter the cycle. Two molecules of CO_2 exit.
- Four pairs of H atoms are removed (three reduce NAD^+ and one – FAD).
- Four pairs of electrons are moved down the chain to reduce ultimately 2 O_2 to water.
- The two CO_2 atoms are *not the same* as in acetyl-CoA (decarboxylation from bottom in α-ketoglutarate); they will be expelled after additional cycles.
- Energetics (presented in biochemistry class) – NADH drives ATP synthesis (about 38 molecules ATP are synthesized per one molecule of glucose).



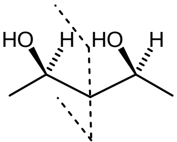
Tartaric acid (dihydroxysuccinic acid, *acidum tartaricum*) is known since antiquity as the cream of tartar (potassium bitartrate) which deposits from wine (lees). The salts are tartrates. The acid occurs naturally in grapes, bananas as L-(+)-, dextrotartaric acid.

Louis Pasteur (1822 – 1895), a French chemist and microbiologist was the first to resolve tartaric acid enantiomers by hand (not easy task now for any chiral compound). He is best known for his remarkable breakthroughs in the causes and prevention of disease. His experiments supported the germ theory of disease, also reducing mortality from fever after child birth, and he created the first vaccine for rabies. To the general public he is best known for inventing a method to stop milk and wine from causing sickness – *Pasteurization* process. He is regarded as one of the three main founders of microbiology. Pasteur discovered the molecular basis for the asymmetry of certain crystals (shown on the right) and for optical activity. *For first time anyone had demonstrated chiral molecules as L- and D-tartaric acid (1849).* Louis Pasteur studied an optically inactive form of tartaric acid that was formed as a deposit in wine barrels during fermentation. When he examined the recrystallized acid under magnifying glass, he noticed that there were two different types of crystals. These types were mirror images of each other. Painstakingly, Pasteur used tweezers to separate the right-handed from the left-handed crystals. He then dissolved each type in water and found that both solutions were optically active, although the original solution was not optically active. He also found that the



solutions rotated the plane of polarized light equally but in opposite directions. With these operations, Pasteur performed the first resolution of a racemic mixture into its constituent enantiomers. He concluded correctly that the optical activity was the result of molecular asymmetry (now termed molecular chirality), and that the original acid was optically inactive because it contained a 50:50 mixture of the enantiomers.

Tartaric acid has third stereoisomer called mesotartaric acid. D- and L-tartaric acids are **enantiomers** because there are not superimposable mirror images but they are **diastereomers** to mesotartaric acid. Diastereoisomers are stereoisomers that are not related as an object and its mirror image. *Diastereomers are stereoisomers that are not enantiomers*. The third isomer is a meso compound which is identical with its mirror image. Meso compounds have an internal plane of symmetry, like a mirror plane, and therefore and not optically active. This plane is indicated in the structures of mesotartaric acid and of meso-2,4-pentanediol.



meso-2,4-pentanediol
four identical groups on the stereogenic centers

Tartaric acid is one of the main acids found in wine and it is added to other foods to give a sour taste. As a food additive, tartaric acid is also used as an antioxidant (E334). Tartrates are other additives serving as antioxidants or emulsifiers. Important derivatives of tartaric acid include its salts, cream of tartar (potassium bitartrate), Rochelle salt or Seignette salt (potassium sodium tartrate, a mild laxative and reagent in carbonyl compounds' analysis used as Fehling solution and in Biuret reagent), and tartar emetic (inducing vomiting, antimony potassium tartrate).